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Protein Fusion & Purification (pMAL™) System

CLONING AND TRANSFORMATION

EXPRESSION

AFFINITY PURIFICATION

FACTOR Xa CLEAVAGE

SEPARATION OF FUSION PROTEIN DOMAIN AND STORAGE

MBP INFORMATION

MISCELLANEOUS

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1. CLONING AND TRANSFORMATION

1.1 What are the differences among the various pMAL vectors?

The pMAL-c, -cRI and -p are the earliest versions of the pMAL vectors. pMAL-c and pMAL-p have a StuI site in the polylinker for cloning blunt-ended fragments. Because the second half of the StuI site codes for proline, if you clone an *Eco*RI fragment into pMAL-c or pMAL-p, the factor Xa site reads IEGRP, and RP won't cut with factor Xa. pMAL-cRI was designed as a short-term solution to fix this problem, by changing the polylinker to code for IEGRI upstream of the *Eco*RI site. The pMAL-c2 and pMAL-p2 vectors are the next generation of pMAL vectors. These vectors avoid the problem with factor Xa cleavege by using an *Xmn*I site instead of StuI. They also have a spacer between *malE* and the factor Xa site which allows some fusions to bind more tightly to the amylose resin, and an M13 origin for making single stranded DNA. The third generation of pMAL vectors is distinguished by the addition of vectors that substitute an enterokinase or Genenase I site for the factor Xa site. These vectors are called pMAL-c2E and pMAL-p2E (enterokinase), and pMAL-c2G and pMAL-p2G (Genenase). The factor Xa versions are now called pMAL-c2X and pMAL-p2X for consistency. This third generation of vectors have a few minor modifications outside the polylinker as well. The NdeI site in the pBR322 origin was destroyed by filling in, and an NdeI site at the ATG of *malE* was added by site directed mutagenesis. This allows a *malE* fusion to be cut out in order to subclone it, for example into a eukaryotic vector. The *NcoI* site in *malE* was destroyed, as was the *Ava*I site in the M13 orgin, making the *Ava*I site upstream of the factor Xa site unique. In all vectors, the "-c" designation refers to cytoplasmic expression, i.e. the signal sequence that directs MBP to the periplasmic space has been deleted. Vectors that are designated "-p" refer to periplasmic expression, and these contain the wild-type *malE* signal sequence.

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1.2 What strain(s) do you recommend as hosts for the pMAL vectors?

The strain we have used most frequently is TB1, #E4122S, which is JM83 hsdR-. There is nothing special about it with respect to the pMAL system, but it has given the best all-around results when considering plasmid stability, expression and purification. We have used a number of other strains successfully for certain proteins. We also use other strains in response to particular problem (for example, see 2.1). One can start with TB1, or one can use whatever competent cells are readily available and then try TB1 or another strain if a problem with expression or purification develops.

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1.3 Can I clone into pMAL vectors just as I would into pUC vectors, i.e. plating my transformants on Xgal/IPTG plates and picking colonies that are white?

No. Even though the β-galactosidase α fragment is the same on the pUC and pMAL vectors, the tac promoter on the pMAL vectors is much stronger than the lac promoter on the pUC vectors. If cells bearing a pMAL vector are induced with IPTG, the cells eventually die. The blue-to-white screen is done by replica plating (or picking and stabbing) onto a master amp plate and an amp Xgal plate containing 0.1 mM IPTG.

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1.4 What is the transformation efficiency of the pMAL vectors?

The pMAL vectors transform about 1/10th as well as pUC and pBR overall. High amp concentration (>100ug/ml) can cause lower efficiencies as well.

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1.5 What primers should I use to sequence the ends of my insert after I clone it into a pMAL vector?

Use the malE primer (cat. #S1237S) on the 5′ side of the insert. If you want to sequence the 3′ junction, the pUC/M13 primers that bind to the lacZ-α region (e.g. #S1224S) will work.

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1.6 What is the transformation efficiency of the lon mutant strains PR745 and ER2508 ?

This strain has a lower transformation efficiency than its parent, PR700. Testing them side by side, PR700 gave about 5 x 10ex6/ug, and PR745 gave ~5 x 10ex5/ug, using cells made competent by the rubidium chloride method. Since the only difference between the strains is the mini-Tn10 in lon, presumably lon-related sickness is the problem, and other protease-deficient strains may be effected as well.

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1.7 What are some of the possible explanations for an inability to clone an insert into a pMAL vector?

The most common explanation for this is technical difficulties with the subcloning. The next most common explanation is that expression of the fusion is toxic to E. coli. The tac promoter induction ratio on the pMAL plasmids is about 1:50, so if the induced level of the fusion is 40% of the total cellular protein, the uninduced level works out to 0.8%. This amount of a protein can be toxic, either because of its function (e.g. a protease) or because of its general properties (e.g. very hydrophobic).

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1.8 How can I obtain the sequences of the pMAL vectors?

pMAL sequences and documents

The sequences are also available by anonymous ftp from vent.neb.com, by Email from info@neb.com, and by fax.

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1.9 Which strand of DNA is packaged when cells carrying the pMAL vectors are superinfected with an M13 helper phage such as M13KO7 (NEB cat #315)?

The strand corresponding to the antisense strand of the *malE* gene is packaged. This is the strand that is complementary to the polylinker sequence shown in the 1998/99 catalog on p. 236. The malE primer (#S1237S) hybridizes to the strand that is packaged by the helper phage.

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1.10 Why am I having trouble cleaving a pMAL-2 vector with the XmnI isoschizomer Asp700 I?

Asp700 I has site preferences, and the *Xmn*I site in the pMAL-2 polylinker happens to be one that Asp700 I cleaves poorly.

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2. EXPRESSION

2.1 When we analyze our fusion protein expression by Western using the anti-MBP serum, only a small fraction of the protein is full-length, and most of it migrates close to the unfused MBP.

It is likely that the fusion protein is degraded, leaving a stable MBP-sized breakdown product. This case is a good candidates for using protease deficient hosts. A list of strains we have available, free with an order or for the price of shipping, can be obtained from NEB. For cytoplasmic expression, the most protease deficient strain is CAG629 (#E4125S) - it is also, however, the most difficult to work with. ER2508 (#E4127S) and CAG597 (#E4123S) are good alternatives. For periplasmic expression, the most protease deficient strain is CAG597 (#E4123S); KS1000 (#E4128S) and UT5600 (#E4129S) might be worth trying as well. The CAG strains are difficult to transform, and often require electroporation to introduce the fusion plasmid.

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2.2 My fusion protein is insoluble; is there anything I can do to get it expressed as soluble protein?

Expressing at a lower temperature is the first thing to try. One can go as low as 15°, by moving a water bath into the cold room. Of course, the cells grow very slowly at these temperatures, so grow the culture at 37° and shift to the low temperature when adding IPTG. One also has to increase the time of induction to compensate for the slower growth - a rule of thumb is 2x for every 7°.

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Some other references for discussion of solubility problems are:

one of the original papers describing how expression at lower temps produces soluble protein: Bishai, Rappuoli &Murphy (1987) J. Bact. 169, 5140-5151

same for an exported protein: Tagaki et al. (1988) Bio/technology 6,

948-950 a method for growing the cells under osmotic stress, which can also help produce soluble protein: Blackwell and Horgan (1991) FEBS Letters 295:10-12

review on methods to make correctly-folded protein in E. coli: Georgiou and Valax (1996) Current Opinion in Biotechnology 7, 190-7.

reviews on insolubility: Schein (1989) Bio/technology 7, 1141-1149 Schein (1990) Bio/technology 8, 308-317 Wilkinson & Harrison (1991) Bio/technology 9, 443-448 Kiefhaber et al. (1991) Bio/techno logy 9, 825-829

reviews on refolding: Zardeneta G; Horowitz PM (1994) Anal Biochem 223(1),1-6 Chaudhuri JB (1994) Ann N Y Acad Sci 721,374-85 Gilbert HF (1994) Curr Opin Biotechnol 5(5),534-9 Schein CH (1991)Curr Opin Biotechnol 2(5):746-50 Kelley RF & Winkler ME (1990) Genet Eng (N Y) 12,1-19 Marston & Hartley (1990) Methods in Enzymology 182, 264-282

extracting from membrane with Sarkosyl: S. Frankel et al. (February, 1991) PNAS, vol.88 pp. 1192-6.

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2.3 When I run my uninduced and induced crude extracts on SDS-PAGE side by side, I don't see an induced band. Why?

There are a couple of possible explanations. Inserts cloned in a pMAL-p2 vector have about a 4- to 8-fold reduced level of expression when compared to the same insert in a pMAL-c2 vector. This often reduces the amount of expression to the point where there is no visible induced band. In addition, some foreign genes are poorly expressed in E. coli, even when fused to a highly expressed carrier gene. Possible explanations are message instability or problems with translation - sometimes it is due to the presence of multiple rare codons in the gene of interest, and in these cases overexpression of the corresponding tRNA can help (Schenk et al., 1995, BioTechniques 19, 196-200). Even in cases where a band is not visible, one can get yields up to 5 or 6 mg/liter of culture.

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2.4 I've cloned my insert, but after SDS-PAGE the only induced band present is the size of MBP2*.

There are two likely explanations for this result. 1) If the protein of interest is in the wrong translational reading frame, an MBP2*-sized band will be produced by translational termination at the first in-frame stop codon. 2) If the protein of interest is very unstable, an MBP2*-sized breakdown product is usually produced (MBP is a very stable protein). The best way to distinguish between these possibilities is to run a Western blot using anti-MBP antiserum (#E8030S). If proteolysis is occurring, at least a small amount of full-length fusion can almost always be detected. DNA sequencing of the fusion junction using the *malE* primer (#S1237S) will confirm a reading frame problem. If the problem is proteolysis, you might want to try one of the protease deficient strains (#E4123S - #E4129S) listed on page 227 of the 2000 New England Biolabs Catalog.

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2.5 What are the possible effects of export (secretion, using a pMAL-p2 vector) on

solubility/stability of the fusion?

Initiating export through the cytoplasmic membrane puts a fusion protein on a different folding pathway - the solubility or stability of a protein is determined by whether this folding pathway leads to a different 3-dimensional structure for the protein. Some proteins, like MBP itself, can fold properly either in the cytoplasm or when exported to the periplasm. However, the normal folding pathway for some proteins is incompatible with passage through the membrane, so the fusion protein gets stuck in the membrane and cannot fold properly; this can lead to degradation (Gentz et al, 1988, J. Bact. 170, 2212-20). Other proteins, especially ones that have multiple disulfide-bonds, only fold properly when exported (the E. coli cytoplasm is a reducing environment, and the proteins that catalyze disulfide bond formation are present in the periplasm)(Bardwell et al., 1991, Cell 67, 581-9). When this class of protein is expressed in the cytoplasm, it may fold improperly and become degraded or insoluble.

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2.6 What is the minimum size of a fragment that can be cloned into pMAL and expressed with the MBP? Can short peptide sequences (about 10 amino acids) be added onto MBP?

You can use the MBP system to express short peptides, and we've heard from several customers that have done it successfully. However, for every 40 mg of MBP (42.5 kDa) one gets about 1 mg of a 10 amino acid peptide (1.1 kDa).

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2.7 Is the N-terminus the only place where a signal sequence leads to export of the protein - is it possible that a protein fused to MBP could contain a fortuitous signal sequence?

It probably would be possible to have some internal region that acts as a signal sequence. It's also possible that hydrophobic regions in the protein lead to association with the membrane, which in turn leads to limited cell lysis during preparation of the periplasmic fraction. Neither one is very common, but perhaps the latter is seen more often.

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3. AFFINITY PURIFICATION

3.1 Much of my fusion protein flows through the amylose column. Is there anything I can do to improve my fusion's affinity for the amylose column?

An MBP fusion protein might not stick to the amylose column because of a low intrinsic affinity or the presence of some factor in the extract that interferes with binding. Factors in the crude extract that can interfere with binding include non-ionic detergents and cellular components that are released during alternative methods of lysis such as lysozyme/sonication or multiple passes through a French press. In addition, cells grown in LB and similar media have substantial amounts of an amylase that interferes with binding, presumably by either cutting the fusion off the column or by releasing maltose that elutes the fusion from the column. By including glucose in the media, expression of this amylase is repressed and the problem is alleviated. A low intrinsic affinity could be caused by an interaction between the protein of interest and MBP that either blocks or distorts the maltose-binding site. Although this may be inherent in the protein of interest, sometimes the problem can be alleviated by shortening or lengthening the polypeptide that is fused to MBP.

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3.2 How many times can I use the amylose column?

The most important variable in determining the useful life of the amylose resin is the amount of time it is in contact with trace amounts of amylase present in the crude extract (cr.. 3.1). Under normal conditions (crude extract from 1 liter of cells grown in LB+0.2% glucose, 15 ml column), the column looses 1-3% of its initial binding capacity each time it is used. If the yield of fusion protein under these conditions is 40 mg. This means that after 3 to 5 runs there would be a decrease in the yield. In practice, we often use a column 8 or 10 times before we notice a significant drop in the yield.

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3.3 What is known about binding in the presence of non-ionic detergents?

What we know is this: 1) some fusion proteins do not bind efficiently (<5% binding) in the presence of 0.2% Triton X100 or 0.25% Tween 20, while other fusions are unaffected, and 2) for a fusion that does not bind in 0.25% Tween 20, diluting the Tween to 0.05% restores about 80% of the binding. We have reports from researchers that purification in the presence of NP40 works, but because of (1) above we don't know how general this is.

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3.4 Can I substitute a different buffer and/or salt concentration in the column buffer?

Yes, we have tried HEPES, MOPS, and phosphate buffers instead of Tris-Cl in the column buffer, at pH's from 7.0 to 7.4 with identical results. NaCl or KCl concentrations of 25 mM to 1 M are also compatible with the affinity purification.

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3.5 I see my intact fusion protein by SDS-PAGE when I run cells boiled in sample buffer, but when I check the crude extract the fusion is degraded.

There are several possible explanations for this. For fusions expressed in the cytoplasm, in many cases most of the degradation happens during harvestand lysis. In this case, harvesting promptly and lysing the cells quickly may help. In other cases, degradation occurs when the fusion protein is exposed to periplasmic or outer membrane proteases (Silber et al., 1992 PNAS USA 89:295-299; Grodberg & Dunn, 1988 J. Bacteriol 170:1245-1253; Sugimura & Higashi, 1988 J. Bacteriol 170:3650-3654).The best strategy in either case is to use a host which is deficient in the offending protease(s) (c.f. 2.1, strains).

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3.6 Can I perform a batch purification using the amylose resin?

Yes, batch purification works well, although it is difficult to wash all the non-specific proteins away as effectively as in a column due to the included volume in the resin. The resin can withstand centrifugation at up to 6000 x g. A good compromise is to load the resin in a batch mode, by incubating with shaking for 2 h to overnight, then pour it in a column to wash and elute. Dilution of the crude extract is not as critical for loading the column by the batch method.

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3.7 Can MBP fusions be purified in the presence of denaturants like urea or guanidine-HCl?

No, MBP's affinity to amylose and maltose depends on hydrogen bonds, that in turn are positioned by the three-dimensional structure of the protein. Agents that interfere with hydrogen bonds or the structure of the protein interfere with binding as well.

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3.8 Is the amylose resin damaged by storage at -20°? When our kit arrived, it was placed at -20°, but I see that the recommended storage temperature for the amylose resin is 4°.

The resin will freeze at -20°, but the performance of the resin is not degraded from one freeze/thaw cycle.

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4. FACTOR Xa CLEAVAGE

4.1 Factor Xa seems to be cleaving my protein at several sites, even though the protein does not contain any IEGR sequences.

The specificity of factor Xa reported in our catalog is as referenced in Nagai and Thogersen (1987) Meth. Enz. 153, 461-481. The basis for this specificity is that the natural factor Xa sites in prothrombin are IEGR (or sometimes IDGR), and many examples of fusions with IEGR are cut specifically. However, proteins can be cleaved at other basic residues, depending on the context (e.g. Nagai et al., 1985, PNAS 82,7252-5; Quinlan et al., 1989, J Cell Sci 93, 71-83; Eaton et al., 1986, Biochem 25, 505-12; Wearne, 1990, FEBS Lett 263, 23-6). A number of the secondary sites (but not all) that have been sequenced show cleavage following gly-arg. We have also seen a correlation between proteins that are unstable in E. coli and cleavage at secondary sites with factor Xa, suggesting that these proteins are in a partially unfolded state. We've tried altering the reaction conditions to increase the specificity, but with no success. It is possibly, however, that adding a cofactor or a substrate analog could change the conformation of the protein enough to block secondary sites (see 4.5 below). We sell pMAL vectors encoding Genenase I sites (pMAL-c2G, #N8068 and pMAL-p2G, #N8069) and enterokinase sites (pMAL-c2E, #N8066 and pMAL-p2E, #N8067) as alternatives.

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4.2 Are there any control substrates for factor Xa?

The Protein Fusion and Purification System comes with an MBP-paramyosin-delSal fusion as a positive control for factor Xa cleavage. It can be obtained separately as #E8051S. Sigma also sells a colorimetric substrate, N-benzoyl-ile-glu-gly-arg-p-nitroanilide (cat # B 7020).

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4.3 How can factor Xa be inactivated?

The best way is 2 uM dansyl-glu-gly-arg-chloromethyl ketone. This compound irreversibly inactivates the enzyme. We get it from Calbiochem, #251700, but there may be other suppliers. We add to a final concentration of 2 uM, then incubate at least 1 min. at room temperature. It reacts with the active site histidine, so it could conceivably react with other sites on the protein of interest, but this is unlikely at the low concentration used. The protease inhibitor chymostatin (60 ug/ml) also works.

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4.4 How can factor Xa be removed from the reaction mix after cleavage?

Factor Xa can be removed by passing the reaction mix over a small benzamidine-agarose column (e.g. Amersham #17-5143-01). When 50 µg of factor Xa is passed over a 0.5 ml column, less than 0.2% of the activity flows through

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4.5 My protein cleaves very poorly with factor Xa. Is there anything I can do to improve cleavage?

We presume that, in these cases, the fusion protein folds so that the factor Xa site is

inaccessible. In theory anything that perturbs the structure might uncover the site. We've tried increasing the temperature, changing buffers and salt conditions, and adding detergents. The only thing that worked was low concentrations of SDS (0.01 to 0.05%; see Ellinger et al., 1991, Virol. 180, 811- 3). Another researcher found that calcium worked - his protein of interest was a calcium binding protein, supporting the idea that anything that might change the conformation even slightly (e.g., a cofactor or substrate analog) could have a dramatic effect.

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4.6 What is the molecular weight and pI of Factor Xa ?

The molecular weight of factor Xa is 42,400 Da. It consists of two disulfide-linked chains, 26,700 and 15,700. On our SDS-PAGE gels they run as 30 kDa and 20 kDa. The calculated pI of factor Xa is 5.09.

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4.7 Is there any thrombin in NEB's factor Xa preparation?

We cannot detect any thrombin in our prep, but our level of sensitivity is not exquisite - we look on an overloaded gel, so we probably would see anything over 1%. The activity of factor Xa on thrombin substrates masks any signal in a proteolytic assay for thrombin (both enzymes cleave after arginine).

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4.8 What is maximum concentration of glycerol that factor Xa can withstand during cleavage?

We have tested factor Xa cleavage in up to 20% glycerol, where it still cleaves at about half the normal rate.

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4.9 How is the rate of factor Xa affected by urea, guanidine hydrochloride and SDS?

The activity of factor Xa on the chromogenic substrate Bz-IEGR-pNA in the presence of these denaturants is as follows:

Urea: In 0.25 M urea, factor Xa cleaves at about 33% its normal rate; at 0.5 M, 25% its normal rate, in 1 M urea, about 10% its normal rate, while in 2 M urea no cleavage is detected.

Guanidine: In 0.25 M guanidine hydrochloride it cleaves at about 15% its normal rate, and in 0.5 M it cleaves at about 5% the normal rate.

SDS: Factor Xa is unaffected by concentrations of SDS below 0.005%. At 0.01% it cleaves at about half its normal rate, and at 0.03% at about one-third normal. At 0.1% and above no cleavage is detected.

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4.10 Can MBP fusions be digested with factor Xa while bound to the amylose resin?

Cutting a bound fusion with factor Xa has been done, by us and in Rawlings and Kaslow, 1992, J Biol Chem 267(6)3976-82. It has two problems that make it less than ideal. First, it takes a lot more factor Xa. With the fusion immobilized, it takes 5% for 24-48h to get decent cleavage. The second problem is that during the incubation, a little of the MBP always seems to fall off the column. This may be because there are trace amounts of amylase bound to the column too, and the amylase liberates enough maltose over time to elute some of the MBP.

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5. SEPARATION OF FUSION PROTEIN DOMAINS AND STORAGE

5.1 In order to re-bind MBP to the column, the maltose must be removed. Can this be done by dialysis?

Dialysis does not work very well to remove maltose from maltose-binding protein. This is a general phenomenon of binding protein/ligand interactions - after the free ligand is gone, ligand that is released from the binding site usually finds another binding site before it encounters the dialysis membrane (Silhavy et al., 1975, PNAS 72, 2120-4). We have determined empirically that binding the fusion to a chromatography resin and then washing away the maltose is much more effective. Another approach is to dialyze vs. buffer in which the protein looses affinity for the ligand (e.g., 2 M urea or pH 4) - this latter approach is not a general one, since some proteins might not be stable in a denaturant or at low pH. We still prefer standard chromatography (e.g. DEAE) as the separation step, since it can separate the factor Xa *and* MBP from the protein of interest. In case MBP co-elutes with the protein of interest, we include a large volume washing step to remove the maltose before starting the salt gradient. This way, the mixture can be run over an amylose column afterward if necessary.

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5.2 How should I store my protein after it is purified?

Most proteins can be stored for at least a few days at 4° without denaturing. For longer term storage, one can either freeze at -70° or dialyze into 50% glycerol and store at -20°. When storing at -70°, aliquot the protein so only the portion to be used must be thawed - repeated freeze/thaw cycles denature many proteins. Freezing at -20°without glycerol may be OK if the freezer is *not* frost-free - the temperature in frost-free freezers cycles enough to cause problems if glycerol is not present.

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5.3 Is there any way of avoiding degradation of protein during storage at 4°?.

Instability of fusion proteins during storage is not generally a problem, but when it is it has always been traced to contaminating E. coli proteases. Try washing the column more before eluting with maltose.

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6. MBP INFORMATION

6.1 What is MBP2*? Is it different from wild-type MBP produced from E. coli?

MBP2* is the protein produced from a pMAL-c2 vector that has a stop codon linker (NEB #S1061S) cloned into the *Xmn*I site. It differs from wild-type MBP by the addition of a methionine at the amino terminus (as do all fusions made in pMAL-c2), the deletion of the last four residues of wild-type MBP, and the addition of the residues encoded by the polylinker.

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6.2 Has the crystal structure of the maltose binding protein been determined?

The references for the crystal structure of MBP are Spurlino et al. (1991), J Biol Chem 266(8)5202-19, and Sharff et al. (1992), Biochem 31, 10657-10663.

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6.3 How much of MBP is dispensable for binding?

The exact region of MBP necessary for binding has not been determined, but the structure indicates that most of the protein is necessary. From the structure, it looks like few if any residues could be deleted at the C-terminus (other than the polylinker

residues, of course). It is possible that some of the N-terminus could be deleted, but so far this has not been tested.
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6.4 What is the Kd , pI and extinction coefficient for MBP2*?

The Kd of MBP for maltose is 3.5 uM; for maltotriose, 0.16 uM (Miller et al., 1983, J Biol Chem 258(22)13665-72.

The extinction coefficient and pI of MBP2* (calculated by computer) are 1.5 (0.1%, 1 cm path) and 4.92, respectively.
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6.5 What is the origin of the MBP region of the pMAL vectors?

The *malE* gene in the pMAL vectors was derived from the *Hin*fI fragment of the *E. coli malB* region. The *Hin*fI fragment lacks the last four amino acids of wild-type *malE*, and of course additional amino acids are added as encoded by the polylinker.
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6.6 Is MBP a monomer or a dimer?

MBP is a monomer. There is one published report that MBP can dimerize in 10 mM Tris-HCl (Richarme, 1982, Biochem. Biophys. Res. Comm. 105, 476-81) but we have not been able to reproduce this result with MBP2*. Gel filtration chromatography in both column buffer and 10 mM Tris-HCl gives a single peak of about 40 kDa.
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7. MISCELLANEOUS

7.1 What is the full reference for the pMAL chapter in "Current Protocols in Molecular Biology"?

Riggs, P. 1992. Expression and purification of maltose-binding protein fusions. *In* Current Protocols in Molecular Biology (F.A. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl, eds.) pp. 16.6.1-16.6.14, Greene Publishing and Wiley- Interscience, New York.
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7.2 Protein Fusion and Purification Strain List

#E4122S TB1	ara del(lac proAB) rpsL (phi80 lacZdelM15) hsdR =JM83 hsdR
#E4121S ER2507	del(malB) zjb::Tn5 del(lac)U169 hsdS20 ara14 galK2 rpsL20 xyl5 mtl1 supE44 leuB6fhuA = RR1 del(malB) del(lac)U169 pro+ fhuA The malE gene is included in the malB deletion, so this strain does not make any MBP from the chromosome. It does not have the lacZdelM15 allele, so it cannot be used for α-complementation (no blue-to-white screen on Xgal). This strain can be transformed with high efficiency, similar to RR1 and HB101.

Protease deficient strains:

#E4127S ER2508 lon::Tn10del16del17 del(malB) zjb::Tn5 del(lac)U169 hsdS20 ara14 galK2 rpsL20 xyl5 mtl1 supE44 leuB6 fhuA

= RR1 lon del(malB) del(lac)U169 pro+ fhuA

The malE gene is included in the malB deletion, so this strain does not make any MBP from the chromosome. This strain can be transformed with fairly high efficiency, about 10x down from RR1 and HB101

#E4124S CAG626 lon lacZam trpam phoam supCts malam rps

CAG626 is ECOk r+m+, so your plasmid has to be modified (i.e., come from an m+ strain such as TB1, JM83, JM107, etc.) in order to get transformants; the transformation frequency is about 100x down from other common strains used for recombinant DNA work, so it helps to use electroporation. (from C. Gross; Baker et al., 1984, PNAS 81, 6779-83)

#E4123S CAG597 rpoHam165 zhg::Tn10 lacZam trpam phoam supCts malam rpsL

rpoHam = htpRam, codes for the heat-shock sigma factor; this strain has a temperature-sensitive amber supressor (supCts), and should be maintained at 30°C. When you induce your expression system (e.g. when you add IPTG), shift the cells to 37o or 42°. CAG597 is ECOk r+m+, so your plasmid has to be modified (i.e., come from an m+ strain such as TB1, JM83, JM107, etc.) in order to get transformants; the transformation frequency is about 100x down from other common strains used for recombinant DNA work, so it helps to use electroporation. (from C. Gross; Baker et al., 1984, PNAS 81, 6779-83)

#E4125S CAG629 lon rpoHam165 zhg::Tn10 lacZam trpam phoam supCts malam rps

rpoHam =htpRam, codes for the heat-shock sigma factor; this strain has a temperature-sensitive amber supressor

(supCts), and should be maintained at 30°C. When you induce your expression system (e.g. when you add IPTG), shift the cells to 37° or 42°C. CAG629 is ECOk r+m+, so your plasmid has to be modified (i.e., come from an m+ strain such as TB1, JM83, JM107, etc.) in order to get transformants; the transformation frequency is about 100x down from other common strains used for recombinant DNA work, so it helps to use electroporation. (from C. Gross; Baker et al., 1984, PNAS 81, 6779-83)

#E4126S CAG748 thi leu lacY tonA supE44 del(lac)X90 dnaJ259 thr::Tn10

This strain has a mutation in the dnaJ gene, which codes for a "chaperonin." This defect has been shown to stabilize certain mutant proteins expressed in E. coli, e.g. mutants of lambda repressor. (dnaJ mutant with linked Tn10; from C. Gross via K. Silber; Straus et al. (1988) Genes Dev. 2:897-904; see also Reidhaar-Olson et al. (1990) Biochemistry 29:7563-7571).

E4128S KS1000 ara del(lac-pro) nalA arglam rif thi1 del(tsp)::kan eda-51::Tn10 /F' lacIq lac+ pro+

This strain is defective in a periplasmic protease, which can cleave proteins that are overexpressed in the cytoplasm when the cells are lysed to make a crude extract. (tsp=tail specific protease, periplasmic protease cleaves the "tail" of a lambda repressor mutant); from K. Silber; Silber et al. (1992) PNAS USA 89:295-9)

E4129S UT5600 ara14 leuB6 azi6 lacY1 proC14 tsx67 del(ompT-fepC)266 entA403 trpE38 rfbD1 rpsL109 xyl5 mtl1 thi1

This strain is deficient in an outer-membrane protease that cleaves between sequential basic amino acids (e.g. arg-arg). It can cleave proteins that are overexpressed in the cytoplasm when the cells are lysed to make a crude extract. (CGSC7092; from B. Bachmann; Elish et al. (1988) J. Gen. Microbiol. 134: 1355-1364; Grodberg & Dunn

(1988) J. Bacteriol 170:1245-1253; Sugimura & Higashi (1988) J. Bacteriol 170:3650-3654).

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